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Effect of Three Varieties of Green Leaves on Sliced Bread

ABSTRACT

The leaves of certain cultivated plants can exhibit a high phenolic content, thereby demonstrating significant antioxidant activity. Phenols, organic compounds found in many plants, possess antioxidant properties that can protect body cells from oxidative damage caused by free radicals. In this study, four different types of bread were prepared: control bread, spinach bread, parsley bread, and tarragon bread (C, T1, T2, and T3) using a 3% partial substitution (PS) of wheat flour with flours from different types of leaves (C, T1, T2, and T3). The physicochemical, antioxidant, and sensory properties of the bread with this 3% leaf addition were evaluated. The results revealed that the inclusion of 3% leaves increased the protein, fiber, and ash values, while reducing the fat content. Additionally, a decrease in crumb lightness and a change in the a* color coordinate were observed. The total phenolic content (TPC) and antioxidant activity (AA) also increased with the addition of these natural ingredients, rising from (51.1 to 101.38 mg GAE/100g) and (15.48 to 50.48 mg AAE/100g) respectively. In terms of sensory evaluation, the bread with 3% spinach flour was the most accepted after the control bread. In summary, the inclusion of 3% leaf flour in the bread influenced its properties, with tarragon being the most notable for increasing fiber, ash, antioxidant activity, and total phenolic content.

Key words: Antioxidants, parsley, polyphenols, spinach, tarragon, Variety of leaves.

INTRODUCTION

Spinach (*Spinacia oleracea*) is a plant cultivated worldwide in tropical and temperate climates. Its leaves provide consumers with a high number of vitamins and minerals, thereby preventing diseases arising from nutritional deficiencies such as anemia (Pardo & Marina, 2010). Notably, it contains flavonoids with anticancer properties that inhibit the cell division of cancer cells in the stomach and skin, similar to the effects of its antioxidants (Herbert & Dispenza, 2016).

Parsley (*Petroselinum crispum*) is an aromatic herb used in various cuisines around the world, although many people are unaware of its numerous health benefits (Monzon, 2023). It is a good source of vitamins K, C, and A, and contains flavonoids and phenolic compounds that inhibit free radicals in the body (Farzaei et al., 2013).

Originating from Southern Europe and Western Asia, tarragon (*Artemisia dracunculus*) is an aromatic herb used in European cuisines. Its composition helps combat flatulence and rheumatism, and it was historically used as a local analgesic due to its eugenol content (Pripdeevech & Wongpornchai, 2012). Its high antioxidant activity and phenolic content make it a potential substitute for synthetic antioxidants (Mumivand et al., 2017).

According to Velez et al. (2012), cultivated plant leaves have a higher phenolic content compared to wild plants. Various parts of plants such as leaves, stems, roots, and seeds are rich in antioxidants, dietary fibers, and essential oils (Das et al., 2012).

As technology and education advance, today's consumers are increasingly aware that food directly impacts their health. This awareness is reflected in their shift in preference towards foods that offer not only satiety but also additional nutritional benefits. Functional foods contain components that enhance health beyond basic nutrition (Skotnicka et al., 2017). In recent years, there has been an increase in research on functional foods with antioxidant properties due to health issues caused by oxygen and free radicals (Jridi et al., 2019).

Bread is a staple food known in various regions of the world. With wheat flour as its main ingredient, it is an essential item on every household table. Its daily consumption helps meet the necessary requirements for a balanced diet (Jing et al., 2019). In a previous study, it was found that adding 3% leaf powder from the Moldavian dragonhead plant to bread maintained acceptable sensory qualities and increased antioxidant capacity. However, this addition did not affect the bread's texture, although it did result in an increase in green color in the crumb and a decrease in its lightness. Additionally, the total phenolic content (TPC) was also influenced (Dziki et al., 2019). Similarly, the addition of parsley to wheat flour reduced the bread's volume and increased its moisture, although these changes had little effect on crumb texture. The crumb color was affected, showing less lightness and more redness due to the parsley. However, partially replacing wheat flour with up to 3% parsley resulted in bread with higher antioxidant activity and acceptable sensory qualities (Dziki et al., 2022a).

The addition of ground leaves in the production of white bread could increase the content of bioactive compounds and improve its nutritional profile. The aim of this study was to evaluate the physicochemical characteristics, antioxidant properties, and sensory quality of white bread enriched with 3% of three different types of leaves: spinach, parsley, and tarragon.

MATERIALS AND METHODS

Raw materiales

For the preparation of bread with green leaves, the inputs (flour, yeast, sugar, butter, salt, and powdered milk) were purchased from the local market, based on their shelf life and technical specifications for each

product. Regarding the green leaf flour (spinach, parsley, and tarragon), it was sourced from Nutrydia Perú.

Bread Formulation and Production

A standard recipe was used to prepare sandwich bread, with a partial substitution of green leaves at 3% (Table 1). These inputs were weighed using a digital scale and placed in a bread-making machine for a basic three-step process (kneading, fermenting, and baking).

Table 1. Bread Formulation with Different Leaves

Componentes	Green leaves			
	Pattern (%)	Tarragon (%)	Spinach (%)	Parsley (%)
Wheat flour	100	97.0	97.0	97.0
Hebs substitution	0.0	3.0	3.0	3.0
Sugar	9.6	9.6	9.6	9.6
Yeast	2.0	2.0	2.0	2.0
Butter	6.7	6.7	6.7	6.7
Salt	1.8	1.8	1.8	1.8
Milk powder	1.6	1.6	1.6	1.6
Water	57.0	57.0	57.0	57.0

Proximate composition análisis

For each bread sample, the following analyses were conducted: Moisture content, using oven drying at 105 ± 0.03 °C for 4 hours; ash content, by calcination in a muffle furnace at 500 °C; fat content, using the Soxhlet extraction method; protein content, using the Kjeldahl method; and carbohydrate content, calculated by subtracting the total from the other components.

Color Properties

Color measurements (CIELAB) in the experiments will be determined using image analysis in ImageJ software version 1.53e. Color indicators L* (Luminosity) and redness degree (s*/b* ratio, where a* represents red-green and b* represents yellow-blue) will be extracted from the RGB information of the ROIs. These data will be used to calculate the color in CIELAB coordinates. For this research, an image acquisition system will be used with: (1) 20 cm walls and matte black internal color; (2) four equidistant white LED lights at 10 cm from the edge; (3) a camera with 52-megapixel image resolution; and (4) a computer with an Intel Core i7 processor and 12GB of RAM. The extracted samples will undergo image acquisition in *.jpg format (Joint Photographic Experts Group).

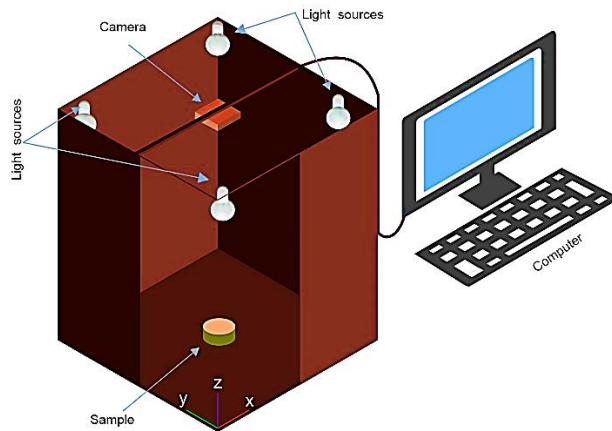


Figure 1. Image Acquisition System

Total phenolic content and antioxidant properties.

Determination of phenolic compounds

For the determination of phenolic compounds, the Folin-Ciocalteu method (Singleton & Rossi, 1965) was used with some modifications. 70% methanol, Folin-Ciocalteu phenol in a 1:5 ratio, 10% sodium carbonate, and gallic acid in a 1:10 ratio were used.

0.4±0.001 g of the sample was weighed into a test tube, which was then placed in a water bath at 60°C. Simultaneously, the sample was agitated in a magnetic stirrer for 30 minutes. Afterwards, the samples were centrifuged at 3500 rpm for 10 minutes to separate the remaining extract, which was decanted with a pipette.

Then, 100 µl of the sample extract was transferred in triplicate to separate tubes, to which 400 µl of Folin-Ciocalteu phenol reagent was added. After a period of 3 to 8 minutes, 2 ml of a 10% sodium carbonate solution was added, the tubes were capped and mixed. The tubes were then placed in a vortex shaker at 300 rpm for 30 minutes before measuring the absorbance in a spectrophotometer, adjusting the wavelengths to 725, 750, and 765 nm.

Antioxidant capacity

The antioxidant activity will be evaluated using the DPPH radical scavenging method according to Brand-Williams et al. (1995) with some modifications. A stock solution was prepared by dissolving 24 mg of DPPH in 100 ml of ethanol and stored at 6°C. Then, the working solution was obtained by mixing 10 ml of the stock solution with 45 ml of ethanol to reach an absorbance of 1.1 ± 0.02 units at 515 nm. For the reaction, 150 µL of the extract was combined with 2850 µL of the DPPH solution and the mixture was shaken for 30 minutes. A spectrophotometer (ThermoSpectronic, Model Genesys 10 UV) with ethanol as a blank was used to measure the absorbance at 515 nm. The antioxidant capacity is expressed as µmol

equivalents of Ascorbic Acid (AA) per 100 g of bread based on a standard curve of Ascorbic Acid (0 – 200 μ M).

Sensory evaluation

It was conducted using a hedonic test with 66 panelists aged 18 to 56, including 39 men and 27 women, who were regular consumers. Each panelist was given a sensory evaluation sheet containing bread samples, including a control and the samples under study. To avoid bias, the samples were cut, bagged, and coded before being given to the panelists. Participants were asked to rate the smell, color, taste, texture, and overall appearance of each sample according to their preferences. The sensory attributes were evaluated using a nine-point hedonic scale, where “1” indicated “dislike extremely,” “5” meant “neither like nor dislike,” and “9” represented “like extremely.”

Statistical Analysis

A Completely Randomized Design (CRD) was used, with four treatments, each with five replicates. Each treatment consisted of a variety of dried leaf flour, designated as T1 (0% dried leaf flour), T2 (3% dried tarragon leaf flour), T3 (3% dried parsley leaf flour), and T4 (3% dried spinach leaf flour). Data on physicochemical, physical, chromatic, and functional properties such as moisture, ash, fat, carbohydrates, crude fiber, protein, area, and total number of pores, instrumental color L^* (white to black), a^* (red to green), b^* (yellow to blue), antioxidants, and polyphenols were evaluated using an analysis of variance (ANOVA). Means were compared using Tukey's test with a significance level set at $P < 0.05$. All statistical analyses were performed using Minitab 17 software.

For the sensory evaluation of smell, color, taste, texture, and overall appearance, the means were compared using statistical analysis performed in Excel 2016.

RESULTS AND DISCUSSION

Proximate analysis

The physicochemical analyses are shown in Table 2; concerning percentage, it was found that all treatments report data below 40% humidity. The Tukey test reveals that there is no statistical significance among the samples. On the other hand, it was found that spinach has a higher protein content (9.5%) than the standard (7.54%), and that the tarragon sample (8.64%) does not significantly differ from spinach in terms of its protein content. The percentage of fat varies among the samples, with the highest being the standard sample (13.42%) and the lowest being the tarragon sample (10.03%). The percentage of crude fiber varies among the samples, with the highest being the tarragon-containing sample (1.71%) and the lowest being the standard sample (0.69%). The parsley sample has the highest ash percentage (1.76%),

while the tarragon-containing sample (1.50%) and the standard sample (1.42%) show the lowest ash percentage.

Table 2. Results of Proximate Analysis. All means are expressed as mean $\pm$ SD (n=3). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

Components	Substitution level			
	Pattern	Tarragon	Spinach	Parsley
Moisture (%)	34.50 $\pm$ 2.85 ^a	30.20 $\pm$ 2.28 ^a	32.84 $\pm$ 6.33 ^a	34.20 $\pm$ 3.6 ^a
Protein (%)	7.54 $\pm$ 1.03 ^b	8.64 $\pm$ 0.32 ^{ab}	9.50 $\pm$ 0.57 ^a	9.30 $\pm$ 1.23 ^{ab}
Fat (%)	13.42 $\pm$ 0.94 ^a	10.03 $\pm$ 1.55 ^b	12.34 $\pm$ 1.83 ^{ab}	11.70 $\pm$ 1.78 ^{ab}
Carbohydrate (%)	42.45 $\pm$ 2.8 ^b	47.92 $\pm$ 1.71 ^{ab}	42.59 $\pm$ 6.23 ^b	41.53 $\pm$ 5.09 ^{ab}
Crude Fibre (%)	0.69 $\pm$ 0.14 ^b	1.71 $\pm$ 0.76 ^a	1.05 $\pm$ 0.47 ^{ab}	1.48 $\pm$ 0.28 ^{ab}
Ash (%)	1.42 $\pm$ 0.04 ^c	1.50 $\pm$ 0.02 ^c	1.67 $\pm$ 0.05 ^b	1.76 $\pm$ 0.08 ^a

The high ash content in bread is influenced by the mineral content of the ingredients used. Parsley leaf flour has a mineral content of calcium and potassium, according to (Saavedra et al., n.d.). The calcium content of parsley leaf is 202 mg/100 grams (National Food and Nutrition, n.d.). The increase in the protein level of the cookies is influenced by the protein content of the raw material used, so the more spinach flour is added, the higher the protein content in the bread (Waseem et al., 2021). According to (Sgarbieri, 1998), the fat absorption capacity of a food is related to the number of non-polar side chains of the proteins that bind to the hydrocarbon chains of the fat. Regarding fiber content, breads substituted with leaves presented a higher fiber content compared to the control bread. This increase was attributed to the high fiber content in the leaf powder used. Additionally, the progressive addition of leaf powder showed a significant improvement in proteins, minerals, and fiber in the cookies compared to the control group (Filip & Vidrih, 2015).

Table 3 shows the results obtained from image analysis. Regarding the number of gas cells, tarragon sample had the highest value of 954.2, followed by the control bread with 894.8, parsley with 867.8, and spinach with the lowest value of 714.6. However, there were no significant differences between the means of tarragon and parsley samples.

The leaves influenced the CIE LAB* color space according to the analysis. In the L* coordinate, brightness decreased as the standard sample value (76.83 $\pm$ 1.62) was higher than spinach (67.38 $\pm$ 3.73). On the other hand, in the a* coordinate, all four treatments had the characteristic green color, with the standard (-4.60 $\pm$ 0.19) having the least coloration and parsley (5.89 $\pm$ 0.48) having the highest value. Finally, for the b* coordinate, all values were in the yellowish hue range, with the lowest value for the control (26.12 $\pm$ 2.39) and the highest for tarragon (42.98 $\pm$ 2.83).

Table 3. Results of the determination of physical properties. All means are expressed as mean $\pm$ SD (n=3). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

Components	Substitution level			
	Pattern 0%	Tarragon 3%	Spinach 3%	Parsley 3%
N ^o . of gas cells	894.8 $\pm$ 3.53 ^{ab}	954.2 $\pm$ 6.36 ^a	714.6 $\pm$ 2.82 ^b	867.8 $\pm$ 2.12 ^{ab}
Area occupied by gas cells (mm)	119.05 $\pm$ 11.5 ^{bc}	117.27 $\pm$ 11.1 ^c	126.01 $\pm$ 15.7 ^a	121.77 $\pm$ 13.6 ^{ab}
Instrumental colour				
L* (White to black)	76.83 $\pm$ 1.62 ^a	70.25 $\pm$ 4.64 ^b	67.38 $\pm$ 3.73 ^c	71.17 $\pm$ 4.01 ^b
a* (red to green)	-4.60 $\pm$ 0.19 ^a	-5.35 $\pm$ 0.48 ^c	-4.87 $\pm$ 0.47 ^a	-5.89 $\pm$ 0.48 ^c
b* (yellow to blue)	26.12 $\pm$ 2.39 ^c	42.94 $\pm$ 2.26 ^a	41.17 $\pm$ 3.29 ^a	42.94 $\pm$ 2.26 ^a

In a similar study Skendi et al., (2019), the numbers of cells and total area determined that the addition of oregano, savory, and thyme at 1% and 2% in enriched breads showed comparable total cell size to the control group, except when dried savory was added, where a notably lower value was observed. The addition of powdered leaves strongly influences the crumb color, which is related to the chlorophyll and carotenoid pigments present in the herbs (Dimov et al., 2018). Similar findings were reported for color changes in cookies supplemented with powdered spinach (Galla et al., 2017). Color change is not necessarily a negative factor in consumer preference for new product development (Zhu et al., 2008).



Figure 2. Bread crumb appearance

Phenolic determination and antioxidant capacity

The total amount of phenolic compounds (TPC) in the four treatments can be seen in Figure 3. It is observed that the bread with partial substitution of tarragon flour at 3.00% showed a significant increase in total phenolic content (101.38 mg eq. GAE/100 g) and antioxidant activity (50.48 mg eq. AA/100 g), compared to the breads with partial substitution of spinach and parsley flour. Likewise, it was observed that the bread with partial substitution of tarragon showed an increase of 98.16% for TPC and 226% for antioxidant activity. On the other hand, the breads with partial substitution of spinach and parsley flours at 3.00% showed total phenolic contents of 81.55 and 74.68 mg eq GAE/100 g, respectively. Also, the

antioxidant activity of these formulations showed values of 33.82 and 44.54 mg eq. AA/100 g, respectively.

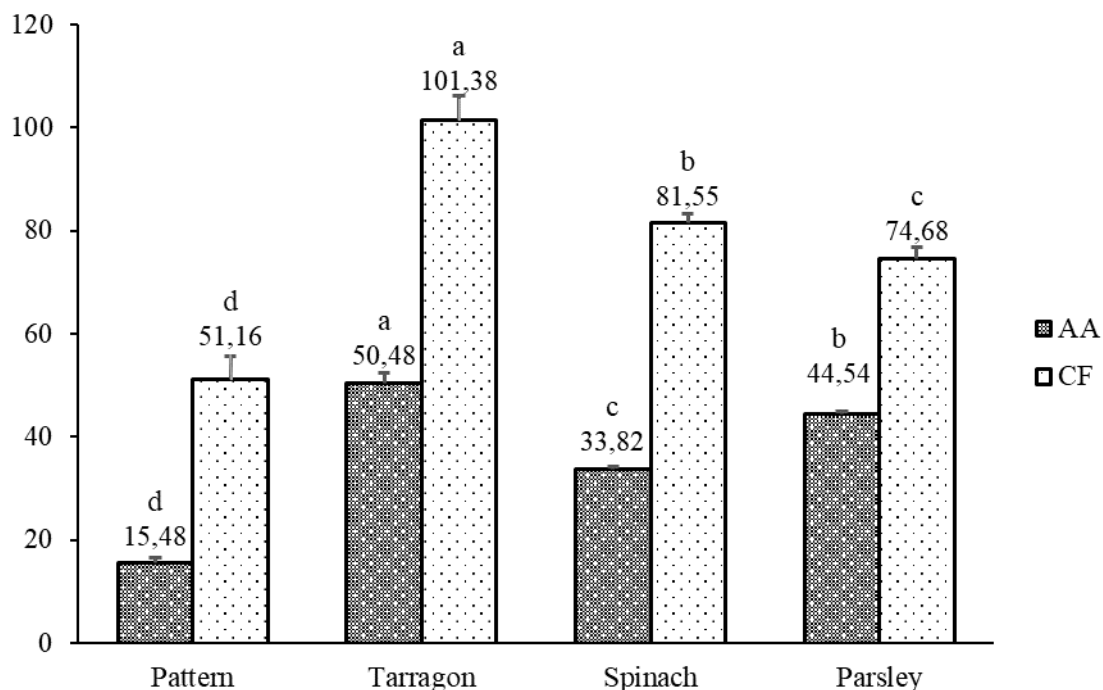


Figure 3. Total phenolic content (mg eq. GAE/100 g) and antioxidant activity (mg eq. AA/100 g) of bread formulations enriched with tarragon, spinach, and parsley flours. The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

The total phenolic content (101.38 mg eq. GAE/100g) and antioxidant activity (50.48 mg eq. AA/100g) of bread with partial substitution of tarragon flour at 3% is higher than the results reported by (Lim et al., 2020), who indicated TPC (10.3 mg eq. GAE/100g) and AA (6.97 mg eq. T/100g) in breads with partial substitution of kenaf flour at 4%, respectively. The antioxidant properties of tarragon could be attributed to the inclusion of phenolic compounds such as luteolin and specifically derivatives of caffeoylquinic acid, which are known to have proven antioxidant activity (Tajner-Czopek et al., 2020). Although high doses of tarragon may offer antioxidant benefits, it is important to note that its application in large quantities can carry health risks. This is due to the known mutagenic and hepatotoxic effects of estragole; however, it has been concluded that its consumption represents a minimal risk to human health (Nesslany et al., 2010). The TPC values are high for parsley as in a study under similar conditions (Dziki et al., 2022b) mentions that by substituting 3% parsley leaf flour in bread, a value of (0.61 mg eq. GAE/g) was obtained, and for spinach, its resulting values were lower when compared to a similar study where TPC (110.56 mg eq.

GAE/g) and AA (210.88 mg eq. T/100g) were found in partial substitutions of spinach leaf flour at 2% and 3% respectively (El-Sayed, 2020; Junejo et al., 2021).

The variation in TPC and AA values in plant leaves can be attributed to a combination of genetic, physiological, and environmental factors, including plant variety, maturity at the time of harvest, and growing conditions (Howard et al., 2002). Also, variations in TPC values may be due to differences in raw materials, processing conditions, and extraction procedures (Mpofu et al., 2006). It is important to consider these factors when interpreting and comparing levels of phenolic compounds in different products.

Sensory evaluation

Table 4 and Figure 4 provides sensory evaluation data for different components and substitution levels in the product, including odor, color, taste, texture, and overall appearance. The average scores for odor, color, and taste were similar for tarragon and parsley, with lower statistical significance. The standard sample was the most significant in these categories, followed by the sample with spinach. The texture of the control stands out with an average of 7.3, indicating a significant difference from the other treatments. Spinach, with an average of 6.5, groups together with the control, indicating no significant difference in score between them. On the other hand, tarragon, with an average of 5.9, falls into a different group, suggesting a significant difference from the control and spinach, although it does not significantly differ from parsley, which has an average of 5.7 and does not share a letter with the other treatments, indicating that it is different from the others.

Table 4. Sensory evaluation on hedonic scale of breads with different green leaves. All means are expressed as mean $\pm$ SD (n=3). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

Components	Substitution level			
	Pattern 0%	Tarragon 3%	Spinach 3%	Parsley 3%
Smell	6.75 $\pm$ 1.17 ^a	4.30 $\pm$ 1.68 ^c	5.68 $\pm$ 1.69 ^b	4.61 $\pm$ 1.74 ^c
Color	7.25 $\pm$ 1.23 ^a	4.96 $\pm$ 1.80 ^c	4.96 $\pm$ 1.47 ^b	4.78 $\pm$ 1.96 ^c
Flavor	7.08 $\pm$ 1.25 ^a	4.57 $\pm$ 1.97 ^c	5.08 $\pm$ 1.61 ^b	4.78 $\pm$ 1.92 ^c
Texture	7.26 $\pm$ 1.16 ^a	5.95 $\pm$ 1.71 ^{ab}	6.55 $\pm$ 1.32 ^{bc}	5.75 $\pm$ 1.95 ^c
General Appearance	7.05 $\pm$ 1.45 ^a	5.57 $\pm$ 2.14 ^b	5.81 $\pm$ 1.41 ^b	5.31 $\pm$ 1.94 ^c

According to Tukey's analysis, significant differences are found between the control, spinach, tarragon, and parsley. The control and spinach do not differ significantly from each other, but both tarragon and parsley are significantly different from the other treatments. Regarding overall appearance, significant differences are also evident between the control and the other three treatments (spinach, tarragon, and

parsley). However, there are no significant differences between spinach, tarragon, and parsley among themselves, as all three significantly differ from the control.

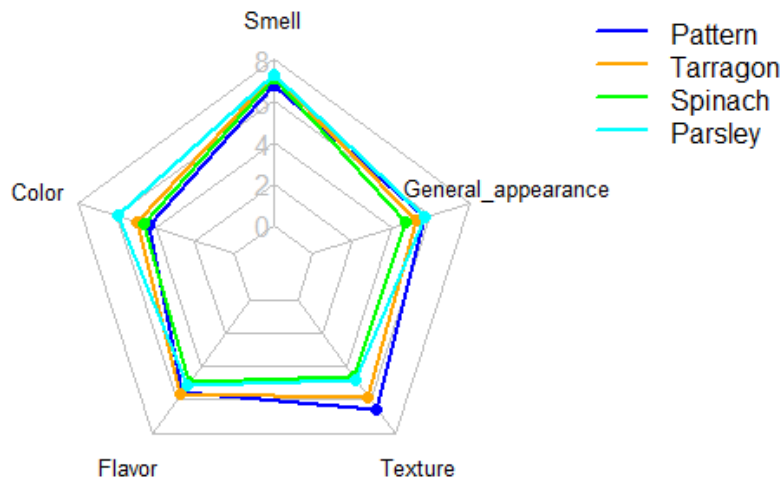


Figure 4. Sensory evaluation results.

Đurović et al., (2020) stated that a 5% increase in nettle leaf incorporation had an impact on the sensory characteristics of bread. These included a darker crumb, distinctive flavor, pronounced grassy aroma, and firmer texture in the bread crumb. On the other hand, Dziki et al., (2022) found that the addition of parsley leaves in the range of 1% to 3% was well received by the evaluated individuals in terms of bread odor and taste. However, as the amount of parsley leaves added beyond this range increased, the panelists no longer showed acceptability in the sensory characteristics of the bread. The low acceptability values of parsley and tarragon in terms of odor and taste may be due to the oil content of parsley; felandrene, α -terpinolene, 1.3.8-p-mentatriene, myristicin, and elemicin (Sany et al., 2022). And tarragon due to being too strong and pungent (Gatfield et al., 2023).

CONCLUSIONS

The antioxidant content and low-fat index make spinach, parsley, and tarragon leaves a good alternative to current foods as they prevent diseases from an unhealthy and disorderly lifestyle. This study revealed that the addition of the aforementioned leaf flours in bread showed an improvement in protein, fiber, and ash compared to the control bread, which can help reduce constipation and cholesterol. High levels of phenols and antioxidant capacity were also obtained, with tarragon standing out among the three leaves, which could inhibit oxidation reactions in the body and reduce cardiovascular and neurodegenerative diseases. The substitution of spinach leaf at 3% had the highest overall acceptance in terms of sensory aspects. This

research could help emphasize the development of baked products with leaf powder substitution as they are healthier than conventional baked goods.

Author contribution

Conceptualization, J.E.-C., L.D.C.-V., A.D.C.-R.; Methodology, J.E.-C., L.D.C.-V., A.D.C.-R., A.C.-S.; Software, J.E.-C.; Validation, L.D.C.-V.; Formal analysis, A.D.C.-R.; Investigation, J.E.-C., L.D.C.-V.; Resources, L.D.C.-V.; Data curation, A.D.C.-R.; Writing-original draft, J.E.-C., A.D.C.-R.; Writing-review & editing, L.D.C.-V.; Visualization, A.D.C.-R.; Supervision, J.E.-C., L.D.C.-V.; Project administration, L.D.C.-V.; Funding acquisition, J.E.-C. All co-authors reviewed the final version and approved the manuscript before submission.

Conflict of interest

The authors declare that they have no conflict of interest.

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